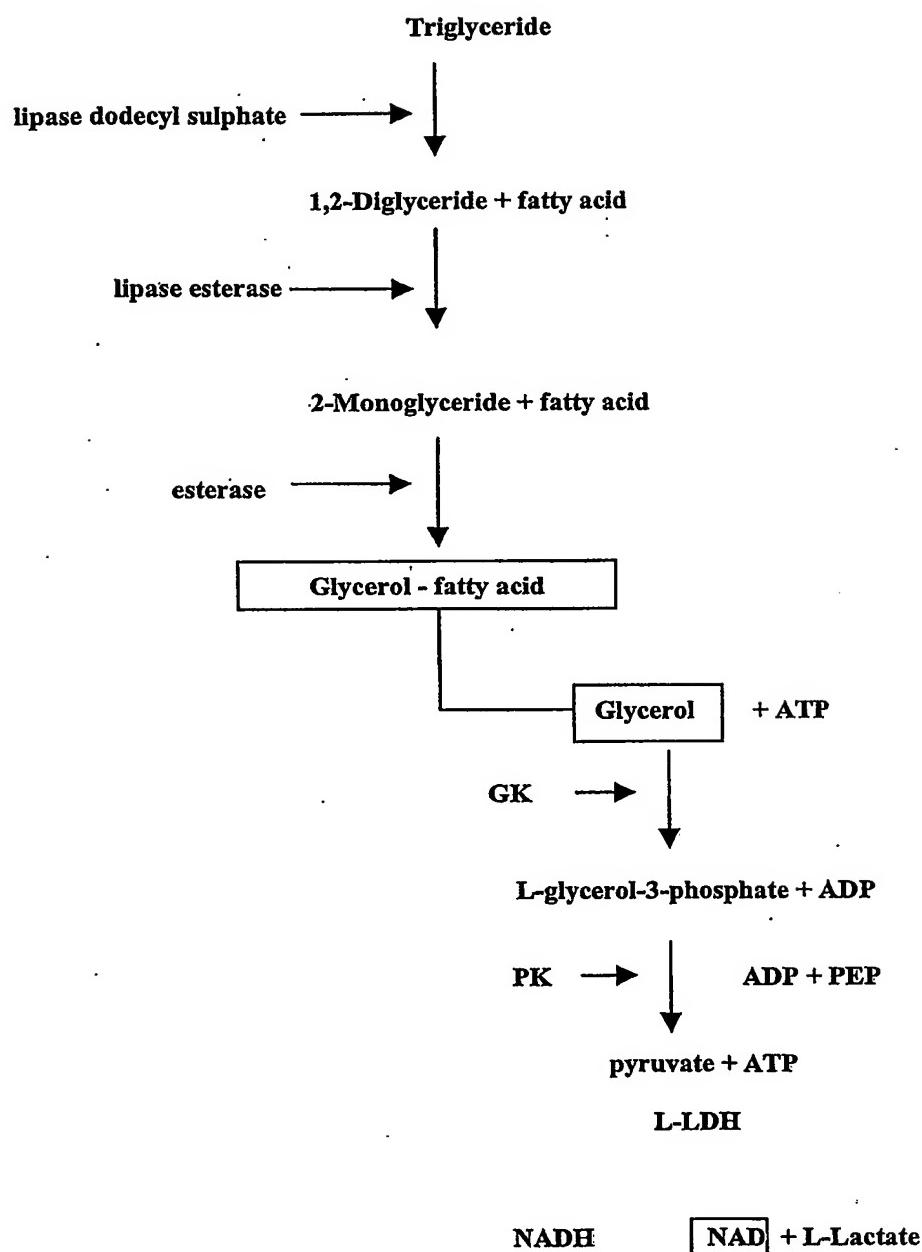
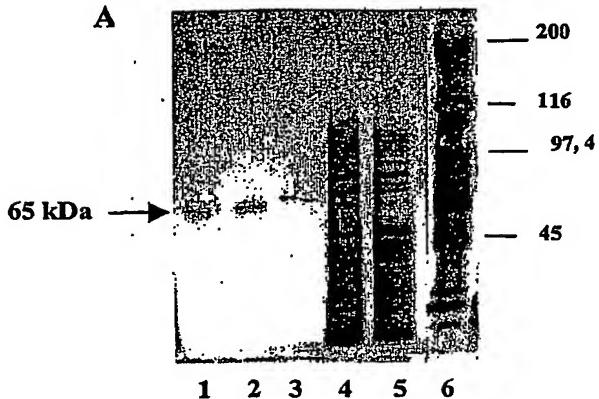
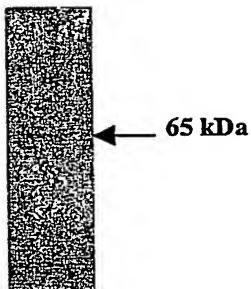


**Fig. 1**

**Fig. 2**

Novel human membrane glycoprotein

**A****B**

**Fig. 3**

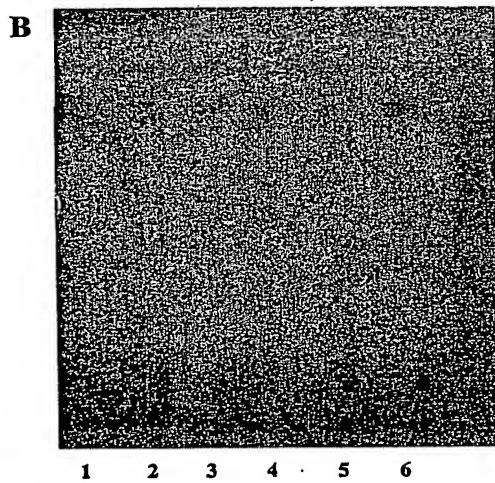
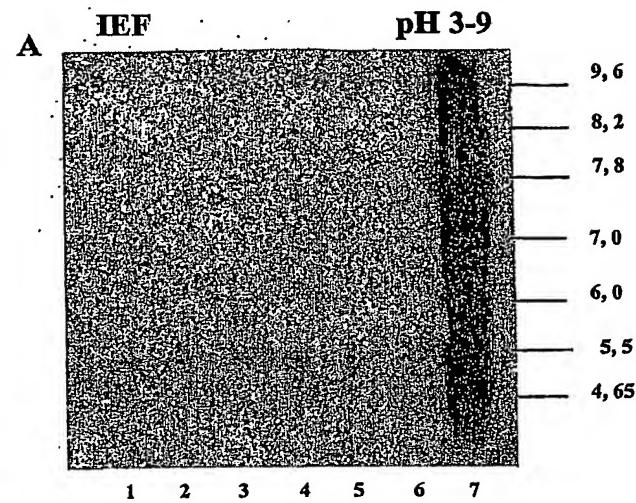


Fig. 4

Peptide A: D-L-V-P-L-E-D-K-V-T-I-L-G-M-T-A

Peptide B: K-L-A-L-S-A-D-D-P-G-F-H-N-F-S-H-Q-R-Q-T

Peptide C: D-Q-Q-T-T-S-H-S-S

Peptide D: V-L-E-I-M-L-P

Peptide E: F-Q-D-E-S-E-A-N-K

Peptide F: M-K-Y-V-N-F-K-F-Y-F

Peptide G: N-L-D-F-M-T-W-G-V-T-K-V-T-Y-I-G-Q-P-T-G-G

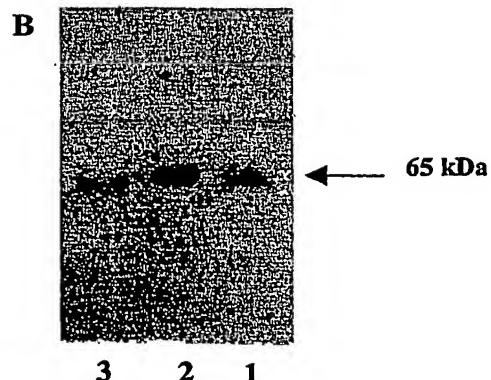
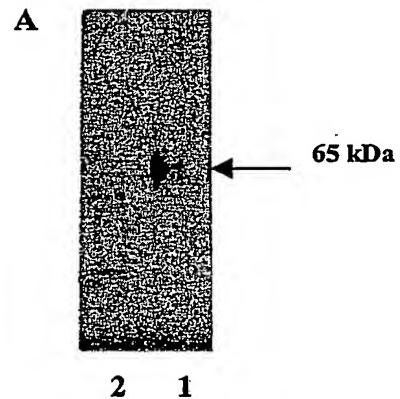
Peptide H: L-L-M-D-N-N-E-A-V-H

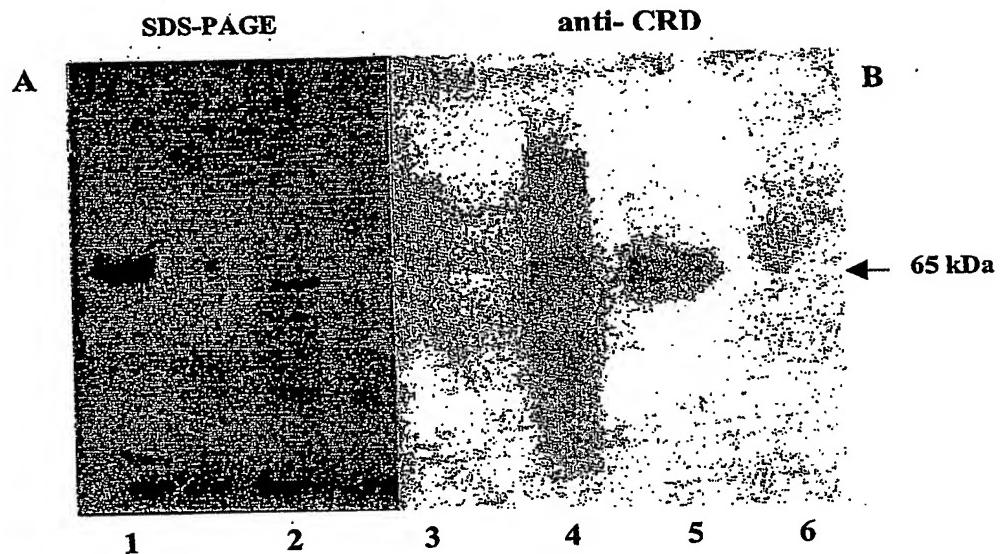
Peptide I: F-D-Q-A-W-A-D-T-A-H-T-W

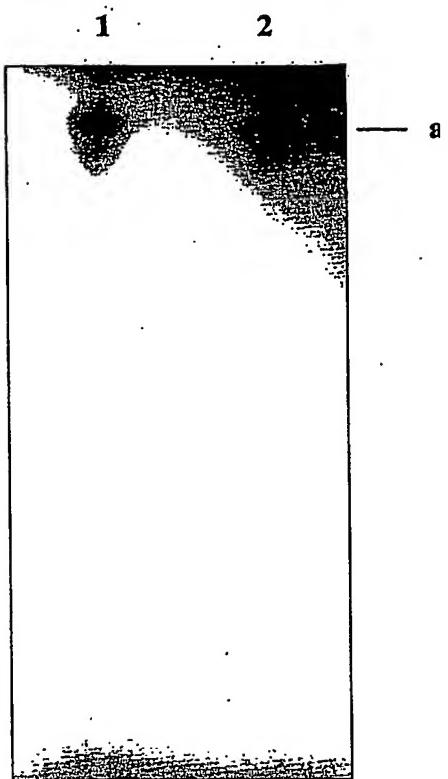
Peptide J: K-L-D-D-I-Q-K-D-M-Y-S-Q-Q-D-T

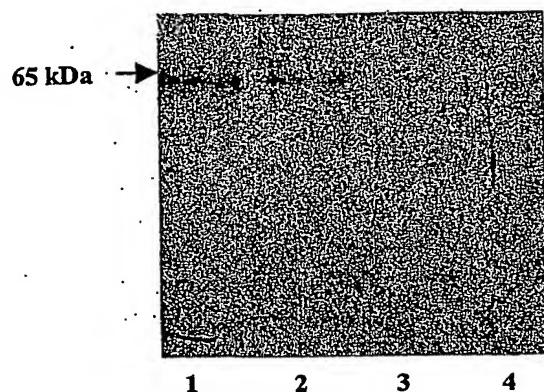
Peptide K: G-V-W-I-M-K-N-Q-I-T

Fig. 5



**Fig. 6**

**Fig. 7**

**Fig. 8**

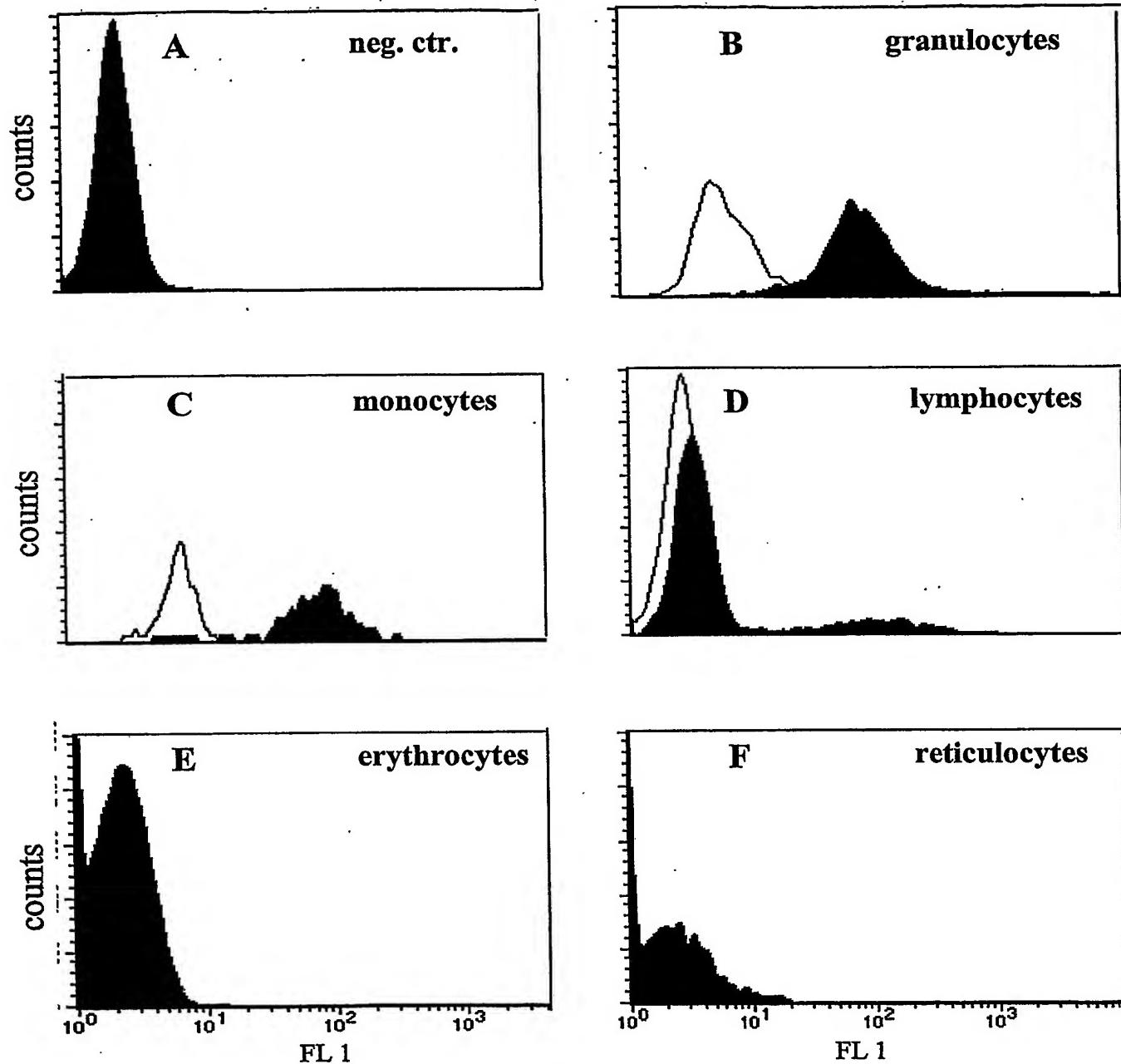


Fig. 9

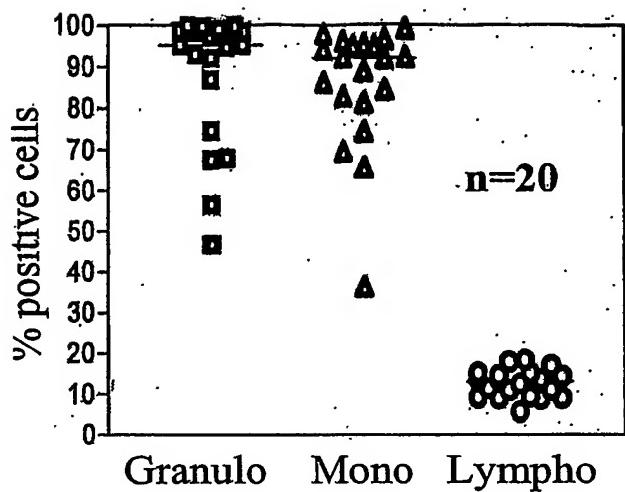
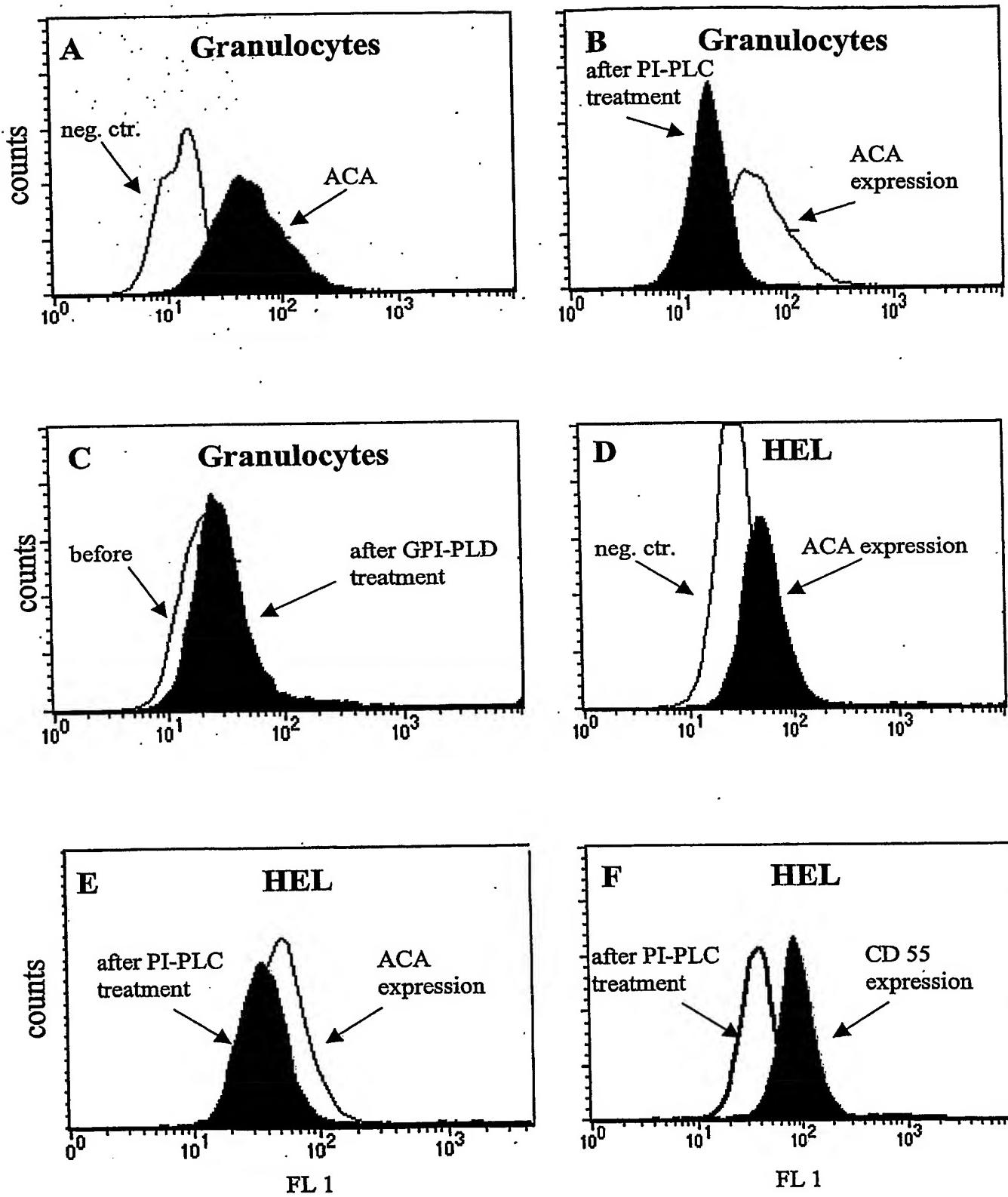


Fig. 10

**Fig. 11**

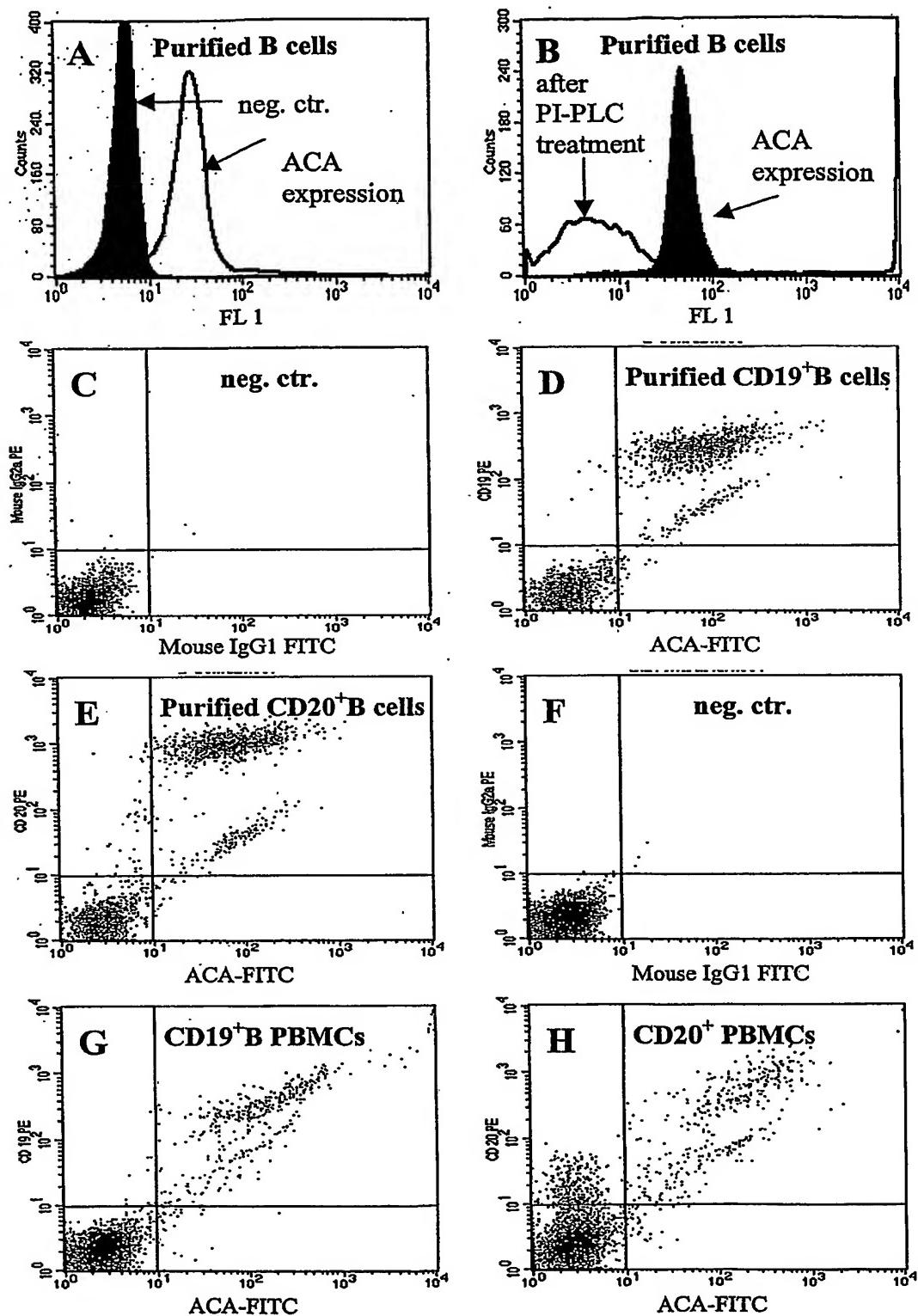


Fig. 12

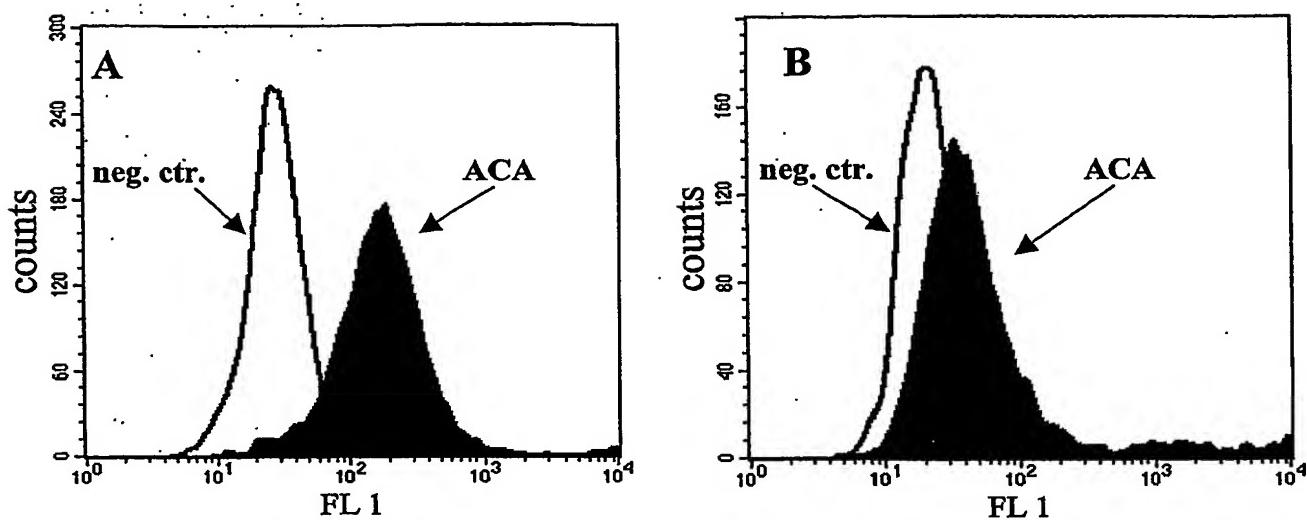
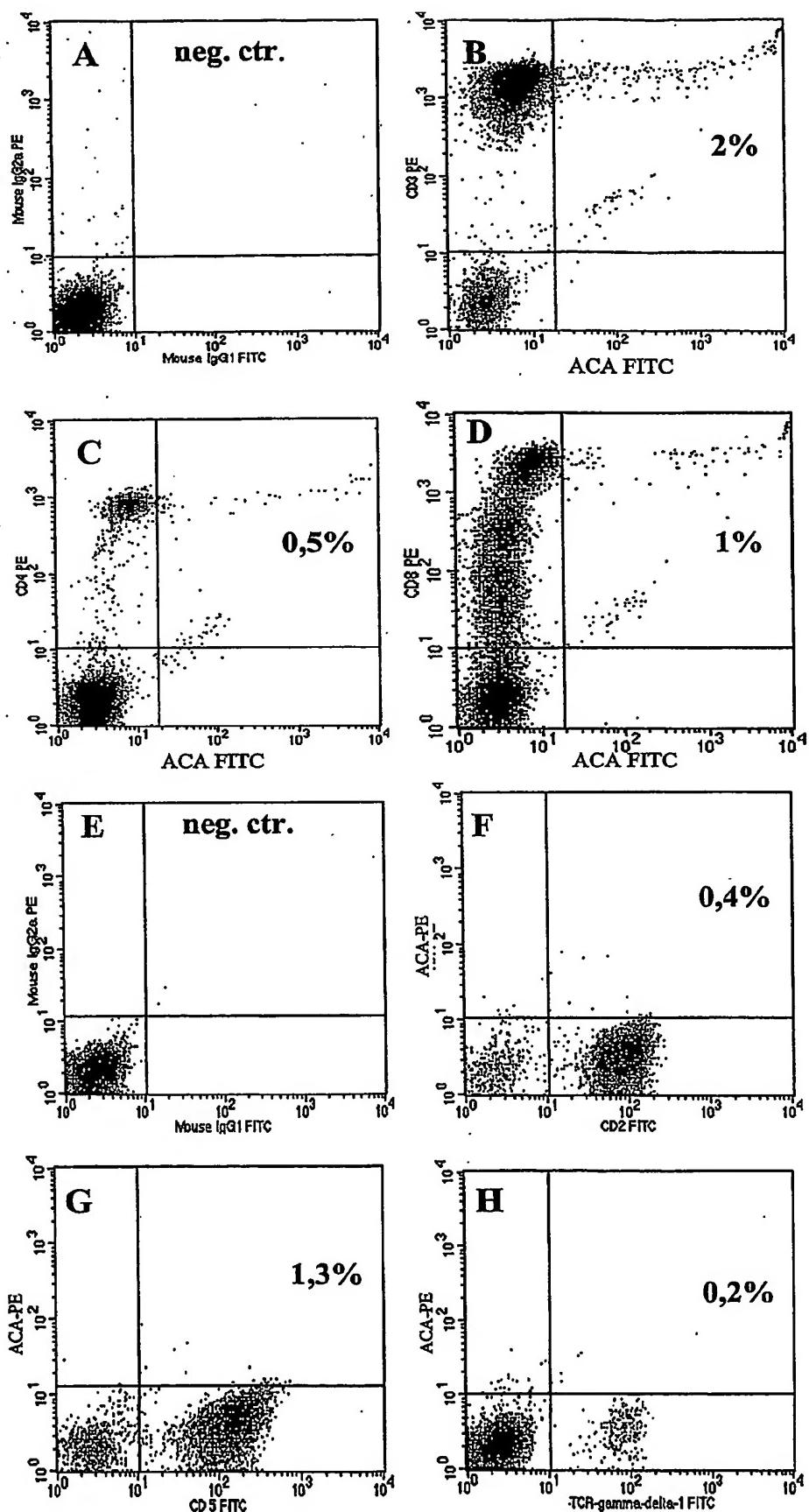


Fig. 13

**Fig. 14**

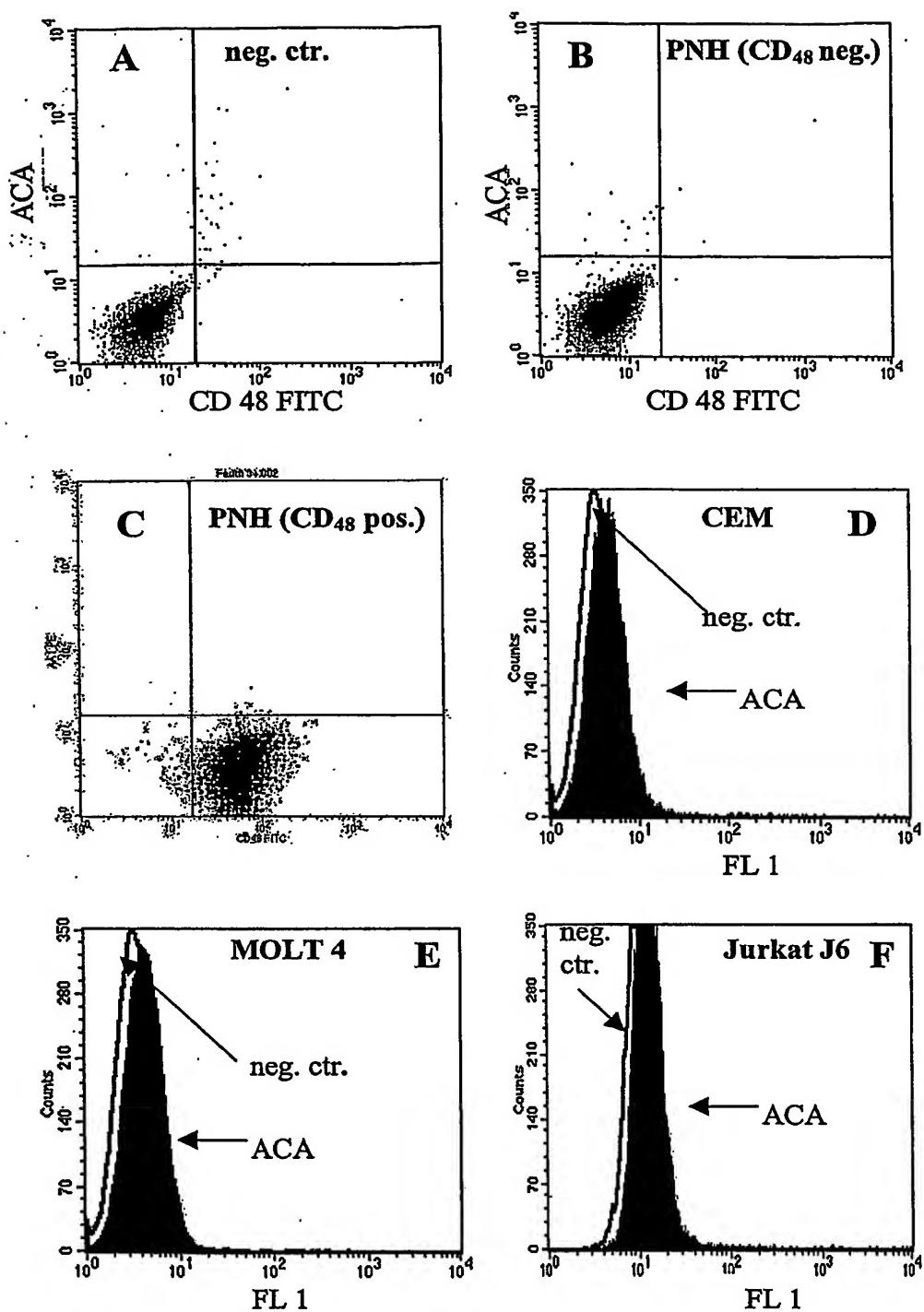


Fig. 15

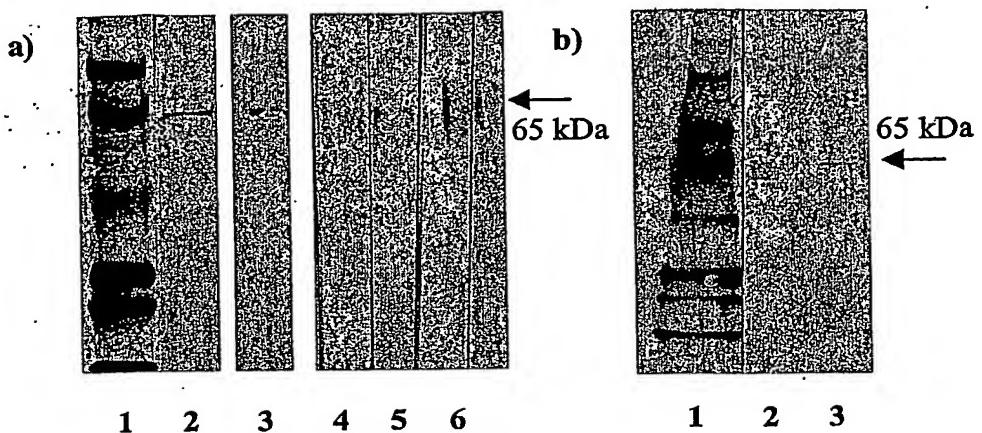


Fig. 16

10/521168

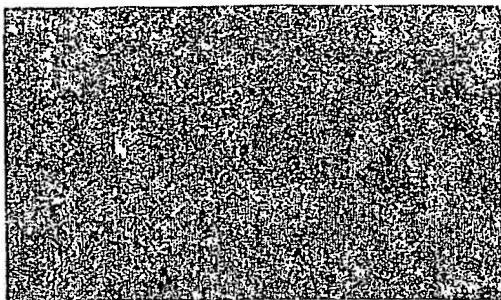
PCT/EP2003/008711

WO 2004/014948

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**Fig. 17**

a)



b)

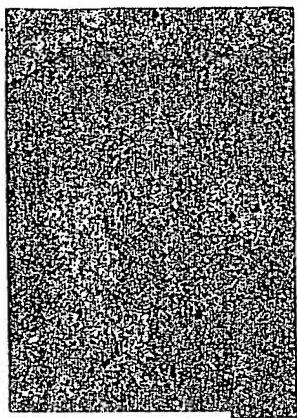


Fig. 17: Frozen sections of normal human skin stained with anti-ACA antibody.

**Fig. 18.**

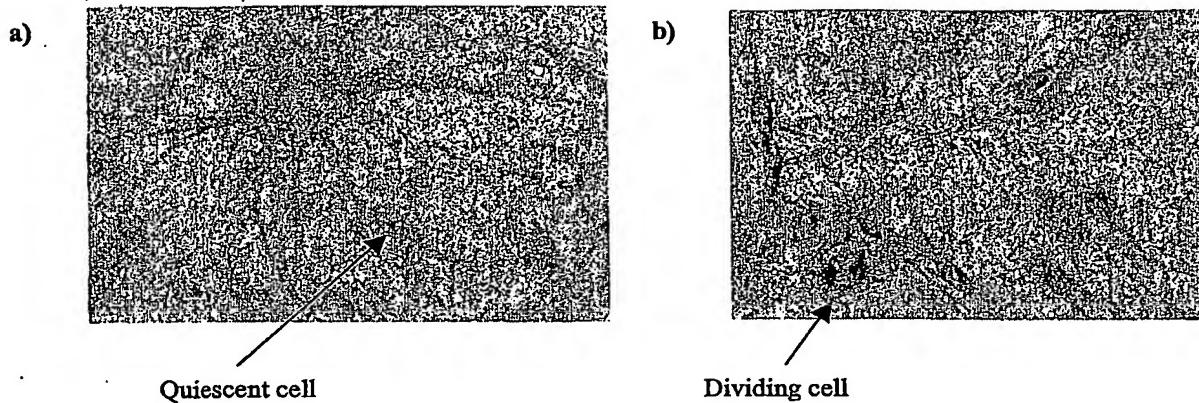


Fig. 18: Normal human cultured epidermal melanocytes stained with anti-ACA antibody.

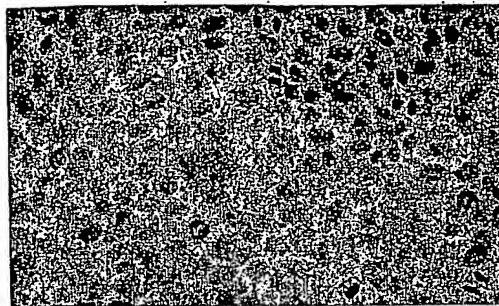
**Fig. 19 a/b****a)****b)**

Fig. 19: Normal human cultured epidermal keratinocytes (a) and keratinocytes cell line HaCaT (b)  
stained with anti-ACA antibody.

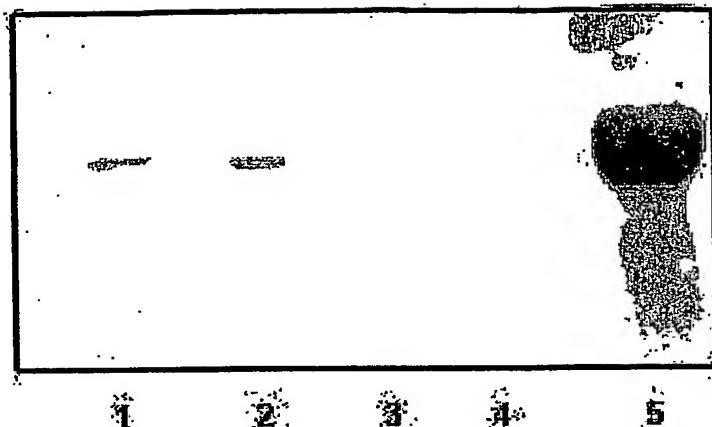
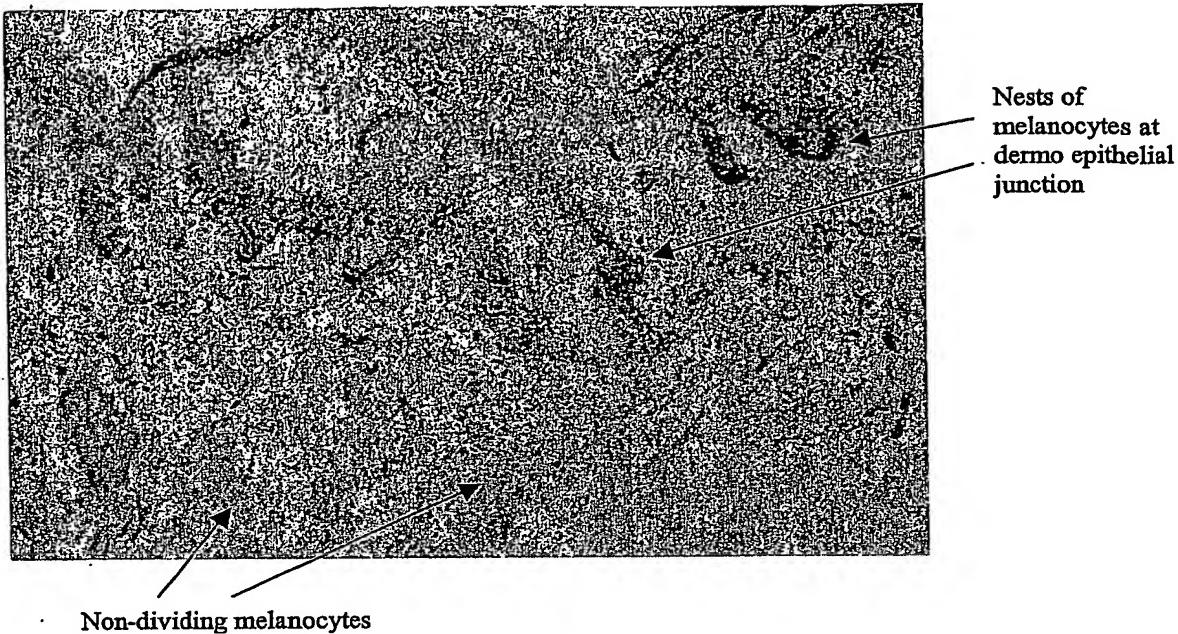
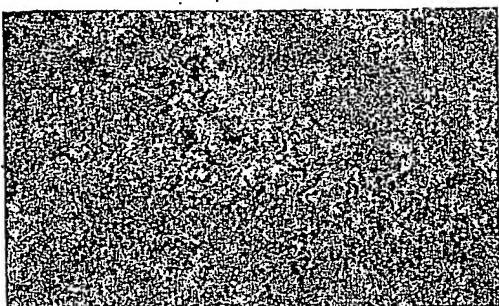


Fig. 19c: Immuno-blot analysis with anti-ACA antibody of cell-free extracts of melanocytes (lanes 1 and 2), keratinocytes (lanes 3 and 4), molecular weight marker (lane 5).

**Fig. 20****Fig. 20: Congenital Naevus**

**Fig. 21**

b)

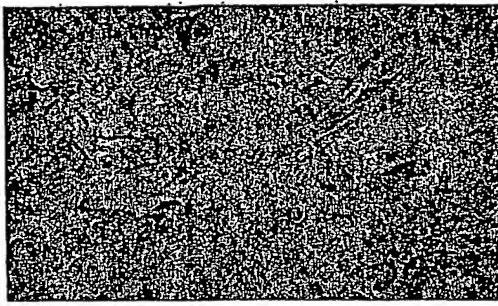
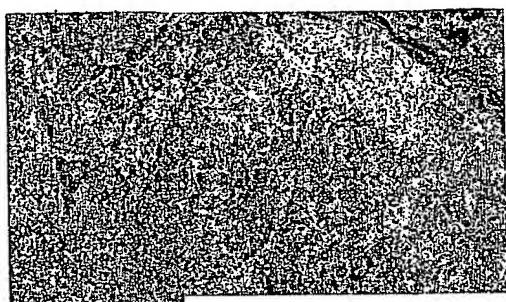


Fig. 21: Frozen sections of human melanoma stained with anti-ACA antibody

**Fig. 22**

a)



b)

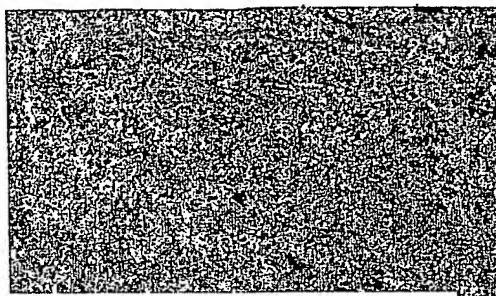


Fig. 22: Melanoma skin metastasis stained with anti-ACA antibody.

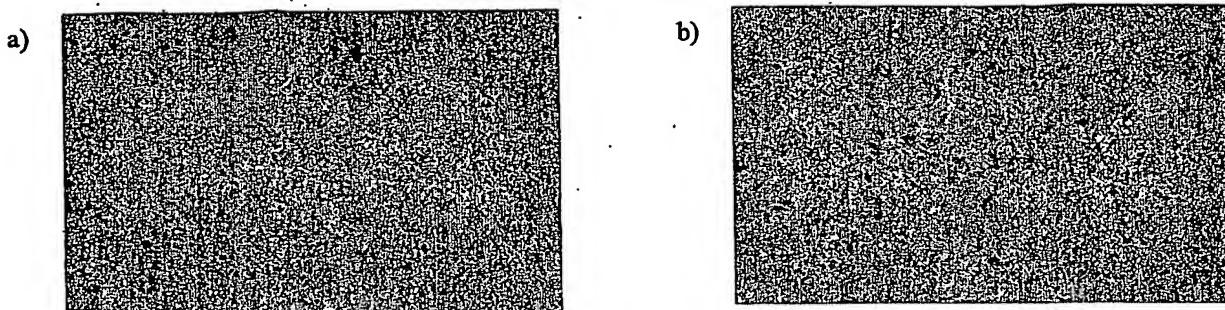
**Fig. 23**

Fig. 23: Frozen sections of human a) basalioma, b) spinalioma stained with anti-ACA antibody.

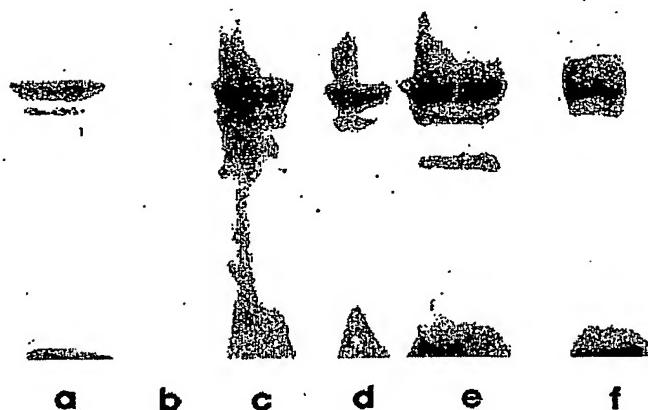
**Fig. 24**

Fig.24: Immunoblot analysis of normal skin and melanoma tumour tissues with anti-ACA antibody:  
Homogenized normal skin (a), non-immune IgG (neg. ctr.) (b), homogenized melanoma tumour tissues  
obtained from different patients (c-f).

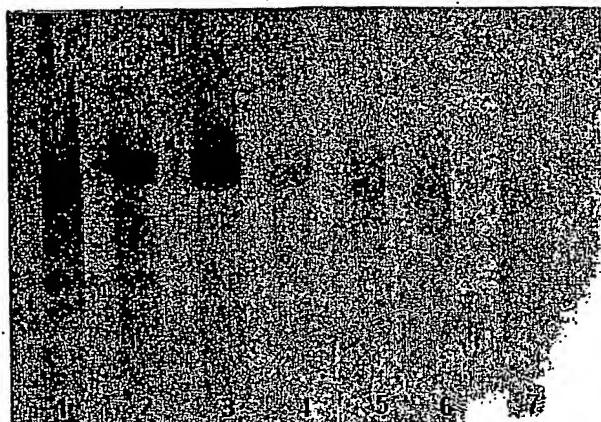
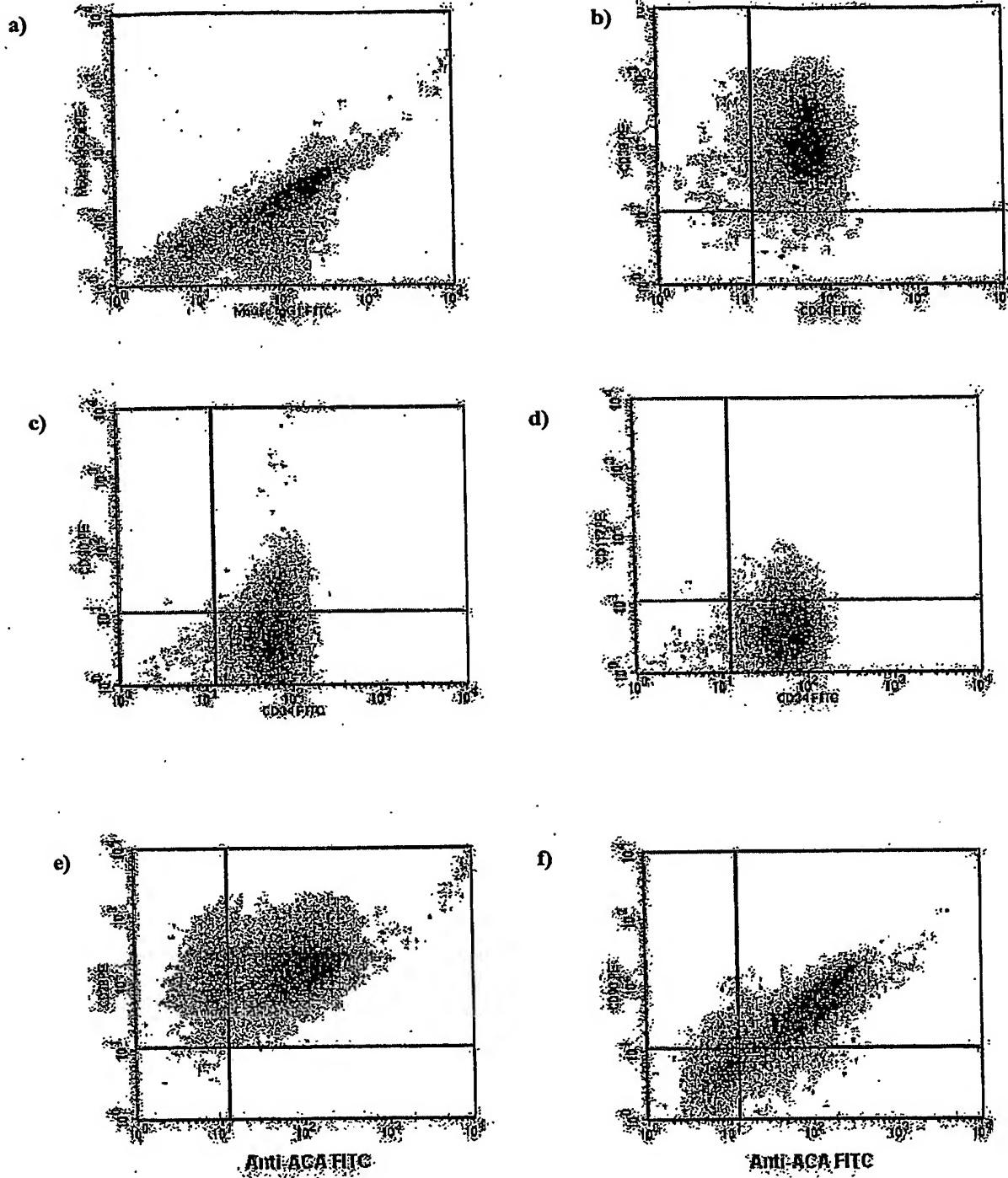
**Fig. 25**

Fig. 25: Immunoblot analysis of homogenized tumour tissues with anti-ACA antibody: Renal (1), Lung (2), Breast (3), Colon (4), Gastric cancer (5), Melanoma (6) and Myeloma (7).

Fig. 26



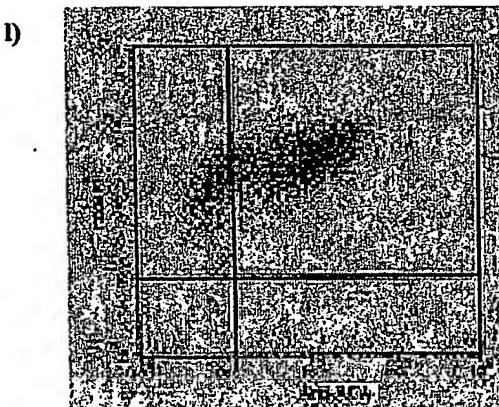
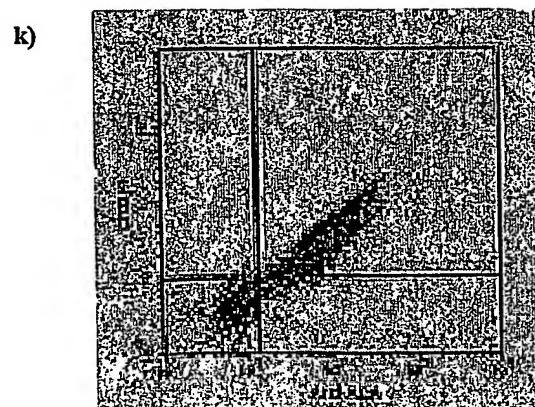
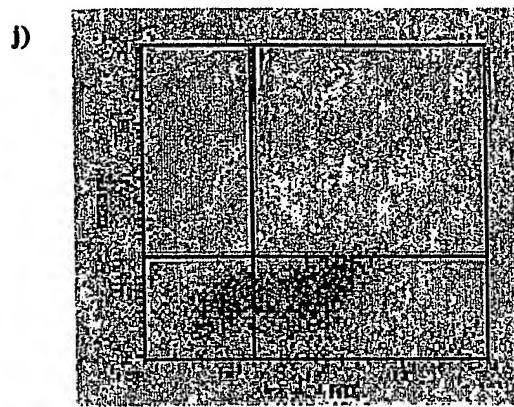
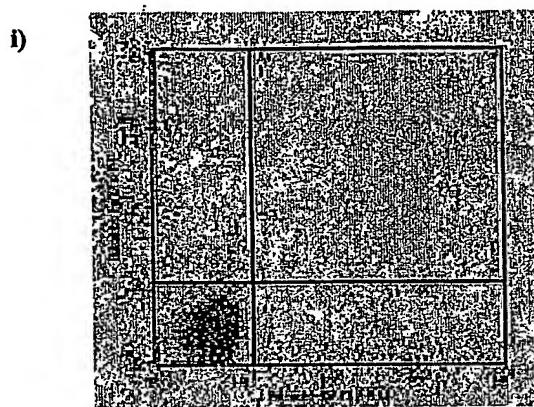
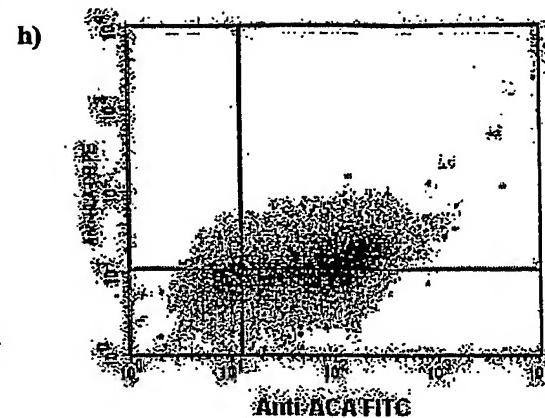
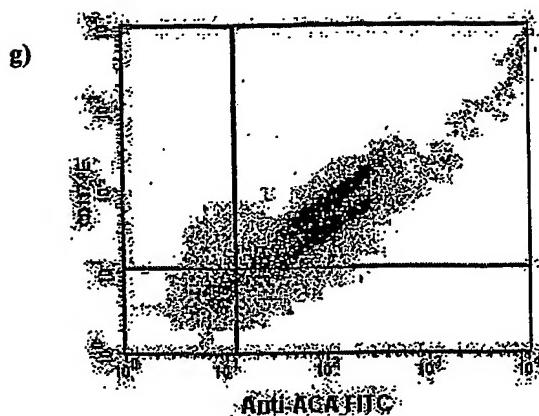
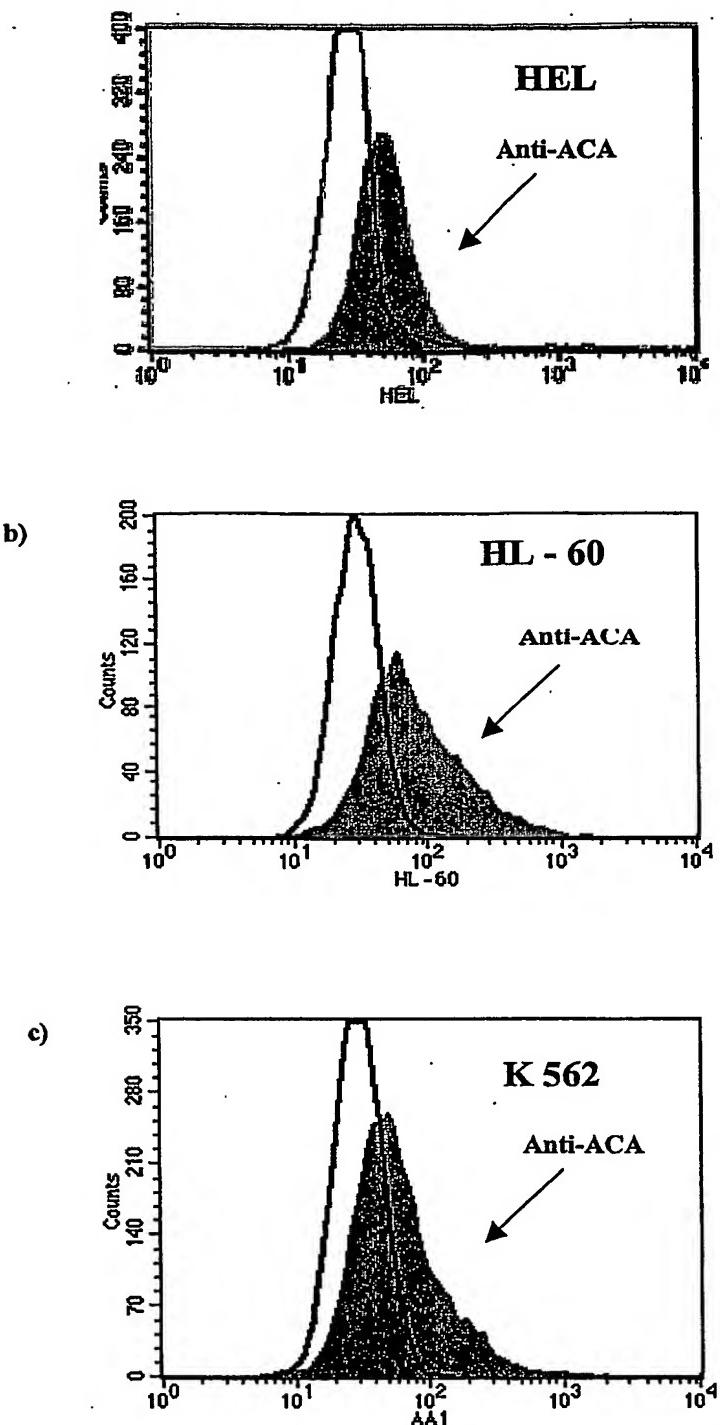


Fig.26: ACA is expressed on stem cell: non-immune IgG (a), CD34/CD38 (b), CD34/CD90 (c), CD34/CD117 (d), anti-ACA/CD38 (e), anti-ACA/CD90 (f), anti-ACA/CD117 (g), anti-ACA/HLA-DR (h), non-immune IgG (i), anti-ACA/CD13 (j), anti-ACA/CD33 (k), anti-ACA/CD34 (l).

**Fig. 27**

**Fig. 27** Flow cytometry analysis of ACA – expression on human leukemia cell lines: (a) erytroleukemia (HEL), (b) promyelocytic erythroleukemia (HL- 60), ( c ) chronic myelogenous leukemia (K562).

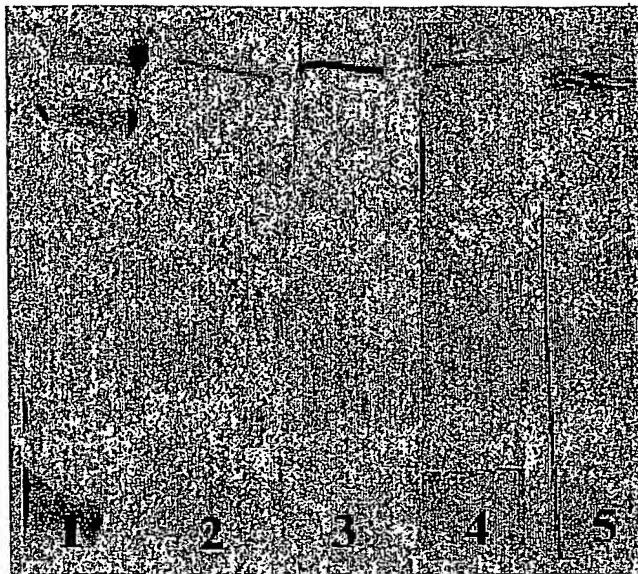
**Fig. 28**

Fig. 28: Immunoblot analysis with anti-ACA antibodies of cell-free extracts of human leukemia cell lines: molecular weight marker (1), human chronic myelogenous leukaemia (k-562) (2), human promyelocytic erythroleukemia (HL-60) (3), human erythroleukemia (HEL) (4), human histiocytic lymphoma (U-937) (5).

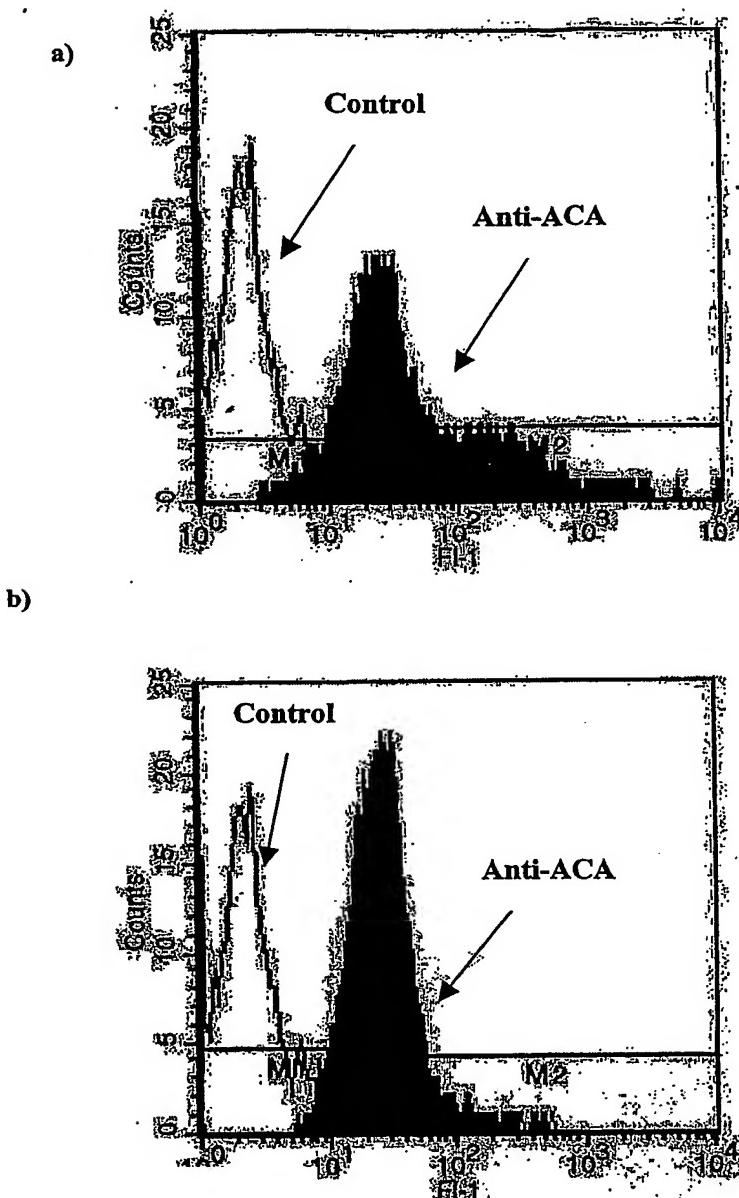
**Fig. 29 a/b**

Fig. 29a/b: Peripheral blood cells of PNH patients were incubated with mouse monoclonal antibodies to ACA plus anti-mouse-IgG FITC and analysed by FACS. After lysis of erythrocytes the granulocytes were gated by FSC/SSC.

**Fig. 29 c**

c)

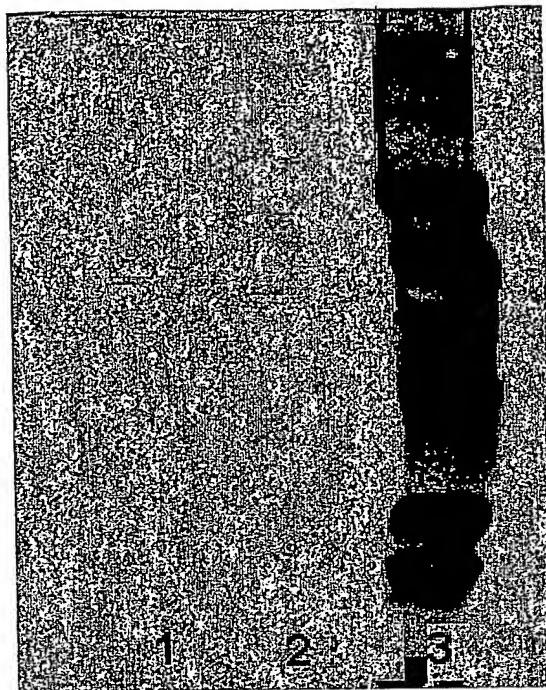


Fig. 29c: Immunoblot analysis with anti-ACA antibody of granulocytes membrane protein fraction obtained from healthy donor (lane 1), versus PNH patient (lane2).

**Fig. 29 d**

d)

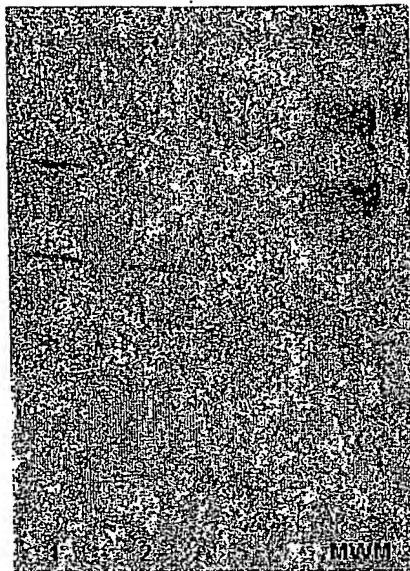


Fig. 29d: Immunoblot analysis with anti-ACA antibody of PNH granulocytes membrane fraction before (lane 1), and after treatment with phospholipase C (lane 2), molecular weight marker (line 3).

**Fig. 29 e/f**

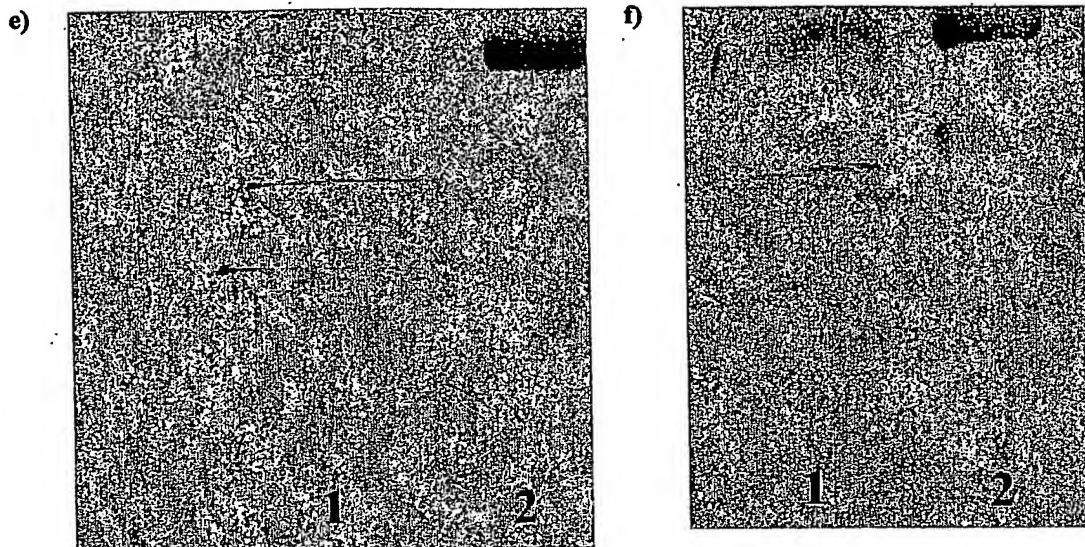


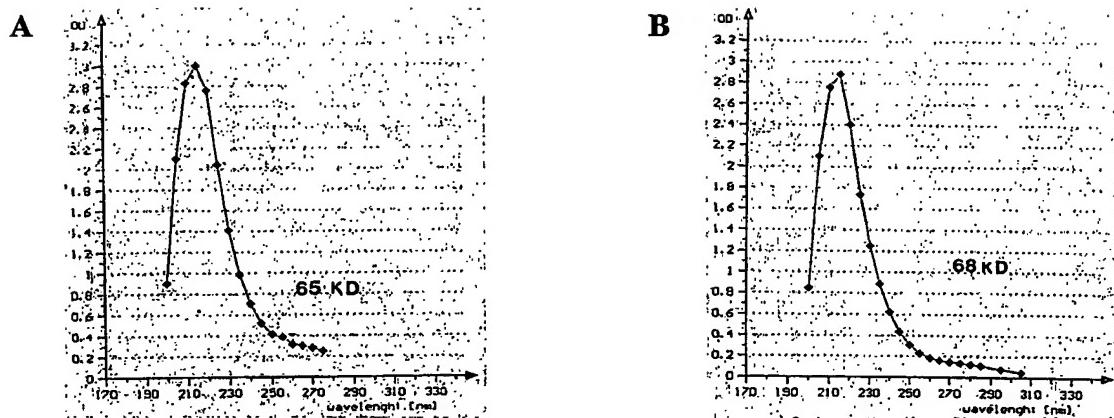
Fig. 29 e,f: Immunoblot analysis of soluble form of ACA in supernatant of granulocytes derived from healthy donor (d) and PNH patient (e) after the action of phospholipase C.

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**Fig. 30**

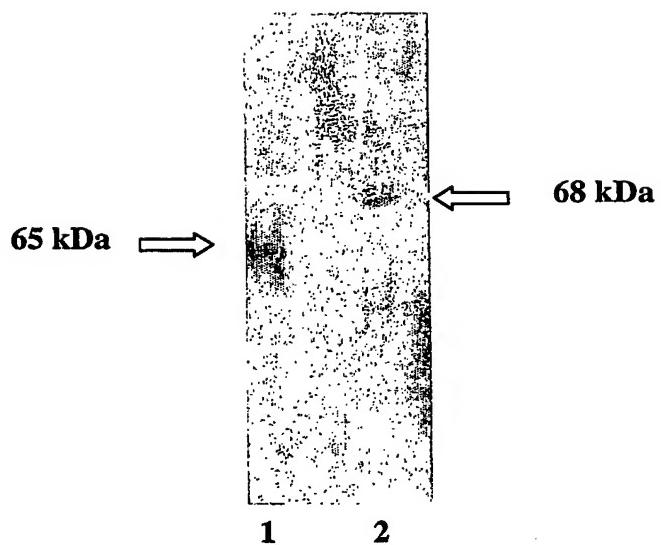
Fig.30 Purification and isolation of ACA proteins. Electrophoresis of purified ACA proteins was performed on 4-15% SDS-PAGE. *Lane 1*, purified main form of ACA protein, molecular mass 65 kD; *lane 2*, purified ACA protein molecular mass 68 kD.

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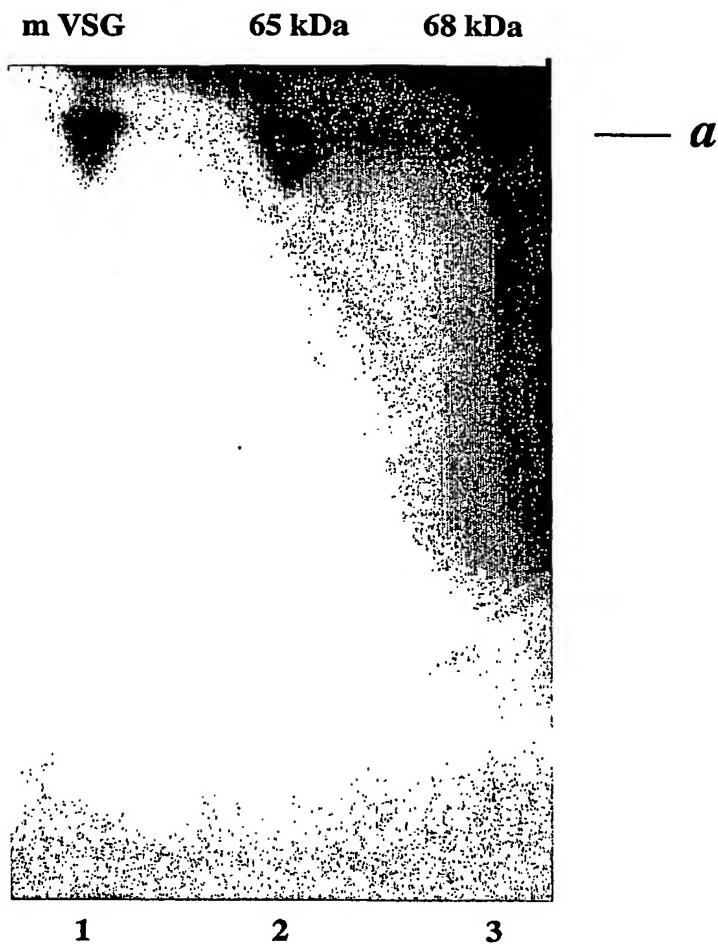
**Fig. 31**

**Fig. 31 UV spectrum of isolated ACA proteins.** UV spectrum of purified two forms of erythrocyte ACA protein was read in Beckman spectrophotometer. A, UV spectrum of purified 65 kD ACA protein; B, UV spectrum of 68 kD ACA protein.

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**Fig. 32**

**Fig. 32 Western blotting with anti-ACA polyclonal antibody**  
Proteins were subjected to SDS-PAGE under reducing condition transferred to nitrocellulose, and revealed with mouse antibody to ACA. *Lane 1*, purified 65 kD erythrocyte ACA protein; *lane 2*, purified 68 kD erythrocyte ACA protein

**Fig. 33**

**Fig. 33 Silica TLC analysis of fragments generated by hydrolysis of anchors.** Samples of [ $^{125}\text{I}$ ] TID-labeled, purified proteins were hydrolyzed with GPI-PLC. The lipid products of this reaction were further hydrolyzed with highly specific lipases. Radiolabeled fragments were extracted and analyzed by TLC. Myristic acid was used as standard. *Lane 1*, commercially available mVSG used as control was labeled, digested with GPI-PLC and further hydrolyzed as described for ACA; *lane 2*, 65 kD ACA protein digested with GPI-PLC and further hydrolyzed; *lane 3*, 68 kD molecular mass form of ACA digested and further hydrolyzed as already described.

Fig. 34

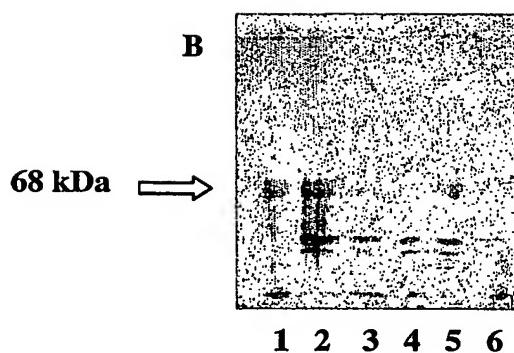


Fig. 34B SDS-PAGE analysis of 68 kD erythrocyte ACA after incubation with PNGase F according to Material and Methods. Lane 1 purified 68 kD ACA after incubation with PNGase F for 0 min; lane 2, purified 68 kD ACA protein incubated with PNGase F for 10 min; lane 3, 30 min; lane 4, 90 min; lane 5, for 150 min; lane 6, overnight.

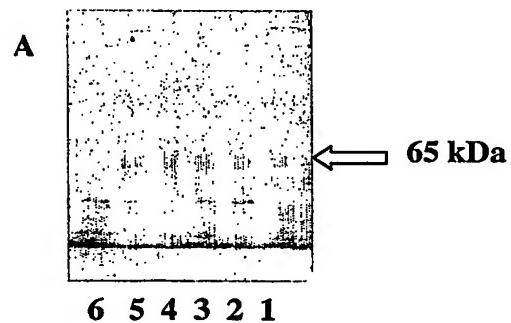
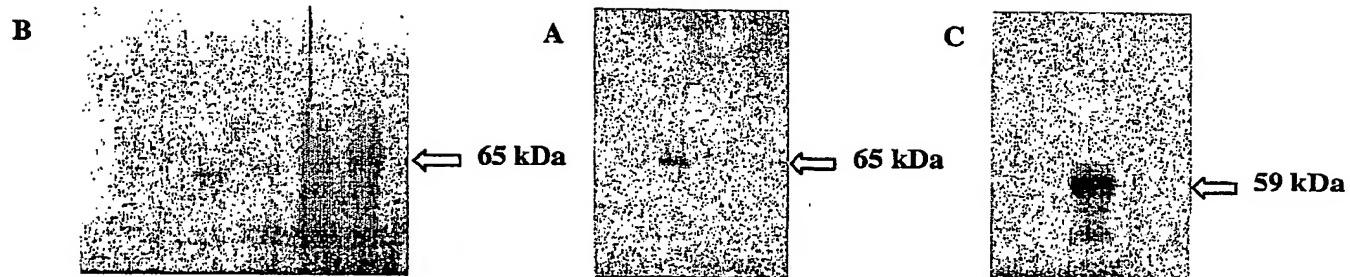


Fig. 34A SDS-PAGE analysis of 65 kD erythrocyte ACA after incubation with PNGase F according to Material and Methods. Lane 1 purified 65 kD ACA after incubation with PNGase F for 0 min, lane 2, purified 65 kD ACA protein incubated with PNGase F for 10 min, lane 3, 30 min, lane 4, 90mins, lane 5, for 150 min, lane 6, overnight.

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Fig. 35



**Fig. 35 Enzymatic and chemical deglycosylation of purified 65 kD erythrocyte ACA.**  
Electrophoresis of purified 65 kD ACA protein and the products of its enzymatic and  
chemical deglycosylations was performed on 4-15% SDS-PAGE. *A*, *lane 1*, purified 65 kD  
ACA. *B*, *lane 1*, purified 65 kD ACA treated with sialidase, *lane 2*, O-glycosidase, *lane 3*,  
PNGase F, *lane 4*, subsequent treatment of 65 kD erythrocyte ACA with sialidase  
O-glycosidase and PNGaseF. *B*, *lane 1* purified 65 kD ACA protein after a chemical  
deglycosylation using TMSF.